CURIOSIS

# APPLICATION NOTE

## Quantification of mitochondrial membrane potential

*Using Celloger® Pro*

## ■ Introduction

Mitochondria serve as the primary organelles responsible for generating energy within cells. In particular, Mitochondrial Membrane Potential (MMP) is considered a key indicator of mitochondrial function, as it is vital for ATP production.[1] Hence, MMP measurement has long been used as a marker for evaluating apoptosis, cell viability, and overall cellular health. Moreover, given that mitochondrial function is recognized to play a crucial role in conditions such as cancer, diabetes, and brain disorders like Alzheimer's and Parkinson's diseases[2], understanding and monitoring MMP become more significant.

Today, numerous fluorescent probes have been developed to visualize mitochondrial potential, including TMRE, TMRM, JC-1, JC-10, Mito-ID, MitoTracker and so on. Among these, JC-1 and JC-10 dyes exhibit changes in fluorescence wavelength depending on the probe's form. In healthy cells with normal MMP, JC-10 accumulates within the mitochondria, forming aggregates that emit red fluorescent wavelengths. On the other hand, in unhealthy cells with declined MMP, such as apoptotic cells, JC-10 scatters across the cytoplasm and converts into monomers, emitting green fluorescent wavelengths.[3] In this application note, we introduce a procedure for evaluating mitochondrial membrane potential using Celloger® Pro.

## ■ Method

HeLa cells were seeded at a density of 3.5 x $10^4$ cells per well in a 24-well plate. Following overnight incubation, the cells were treated with carbonyl cyanide 3-chlorophenylhydrazone (CCCP) (Sigma, C2759-100MG) at concentrations of 0.5, 1, 2, 4, 6, 8, 10 and 20 μM for 20 minutes. After a wash with HBSS buffer, the cells were stained with 10 μM JC-10 (AAT bioquest, 22204) solution in the dark for an hour. Finally, images were taken with Celloger® Pro, automated live cell imaging system with a 2X lens.

## ■ Result

We observed changes in fluorescence resulting from depolarization using JC-10 dye and carbonyl cyanide 3-chlorophenylhydrazone (CCCP), which acts as an uncoupler of oxidative phosphorylation. For quantitative comparison, we captured green and red fluorescence images using the Celloger® Pro with 2X lens for a wide field-of-view. And then, we employed analysis software to measure the intensity changes. As the concentration of CCCP increased, the membrane potential depolarized, leading to a decrease in red intensity and an increase in green intensity. (Fig. 1A). This trend persisted until a concentration of 20 μM was reached, at which point red intensity became almost negligible, causing the cells to appear entirely green. Figure 1B, captured with the Celloger® Pro using a 2X lens and cropped for magnification, enables the observation of red and green fluorescence distribution. The graph in Figure 1C, which depicts the green/red intensity ratio in relation to CCCP concentration as a dot plot, showed a gradual ascending pattern suitable for polynomial regression curve fitting.

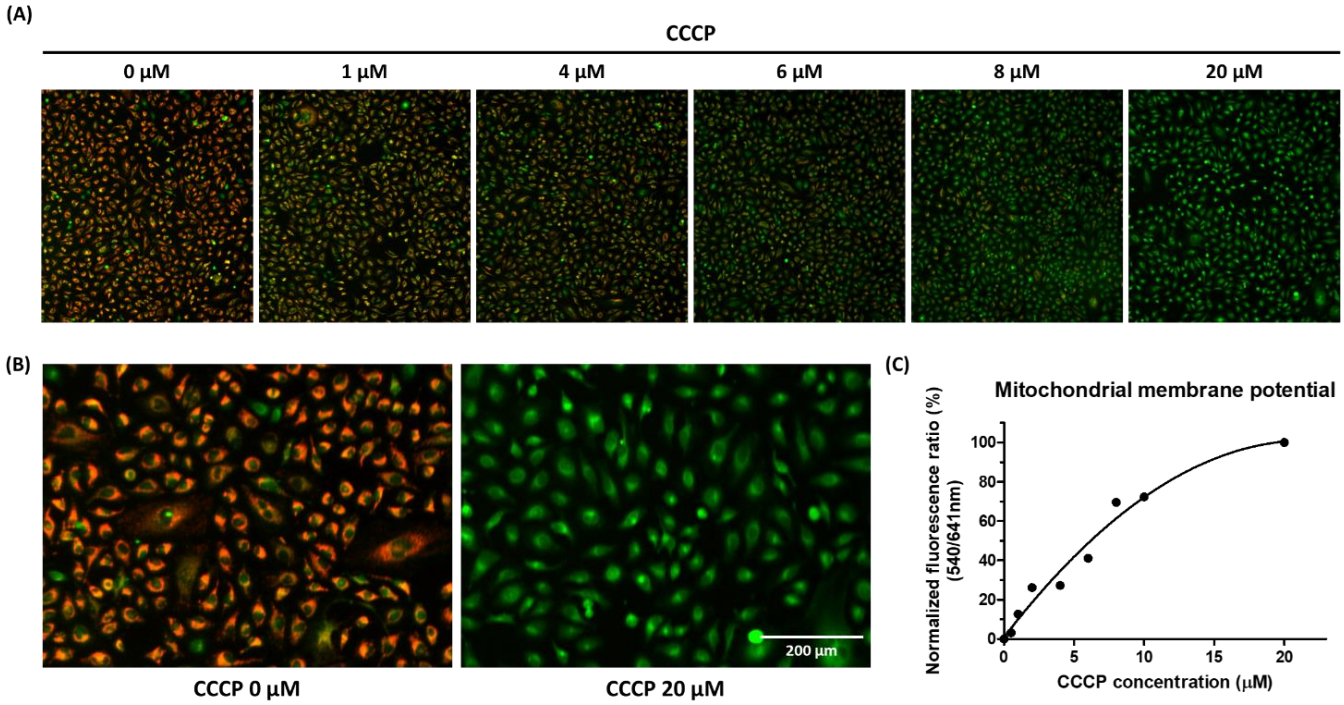


**Figure 1. Mitochondrial dysfunction depending on CCCP concentrations.**

(A) The intensity of both green and red fluorescence varied with different CCCP concentrations. (B) In the control group, strong red dot-like signals were observed, whereas CCCP-treated cells displayed a prominent spread of green fluorescence throughout the cytoplasm. (C) The relationship between the green/red ratio and CCCP concentrations is modeled using a second-order polynomial curve ($R^2$=0.97).

## ■ Conclusion

Mitochondrial activity in cells is intricately regulated in response to intracellular conditions, including factors such as MMP, which can vary depending on the stage of the cell cycle.[4] Therefore, it is important to analyze it within sufficiently wide areas to consider these variations, but collecting images from multiple regions is such a laborious task. Additionally, dyes like JC-10, which display two colors depending on their molecular forms, require individual detection using two different fluorescence channels. We successfully addressed these challenges by utilizing the Celloger® Pro, which offers features such as dual color fluorescence and bright-field imaging. Moreover, it efficiently captures images from multiple positions by automatically moving the camera.

**Reference**


1. Zorova, Ljubava D., et al. "Mitochondrial membrane potential." Analytical biochemistry 552 (2018): 50-59.

2. Bazhin, Arkadiy A., et al. "A bioluminescent probe for longitudinal monitoring of mitochondrial membrane potential." Nature chemical biology 16.12 (2020): 1385-1393.

3. Sivandzade, Farzane, Aditya Bhalerao, and Luca Cucullo. "Analysis of the mitochondrial membrane potential using the cationic JC-1 dye as a sensitive fluorescent probe." Bio-protocol 9.1 (2019): e3128-e3128.

4. Hirusaki, Kotoe, et al. "Temporal depolarization of mitochondria during M phase." Scientific Reports 7.1 (2017): 16044.